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**Healthcare Studies 2(1) (2024): 33 –45**

Research Article

# Xylene-Free Histoprocessing using an Alternative Method: Effect of Microwave Temperature

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**Abstract:**
The mechanism of microwave histoprocessing is based on the rapid dehydration of previously fixed tissue using microwave energy to heat the reagent (alcohol) to a temperature below its boiling point. Isopropanol further dehydrates the tissue and prepares it for paraffin infiltration. The present study addresses the negligence of the effect and efficiency of microwave energy in histological techniques. This alternative method has more than a few merits over conventional methods from the viewpoint of laboratory staff. It also has some environmental benefits as it eliminates xylene in tissue processing. Processing was performed at 315K for dehydration and 4523K for infiltration. Clearing agents were not employed because the temperature of the final paraffin step increases the evaporation of alcohols from the tissue. Subsequently, tissues were embedded sectioned and stained using the traditional technique.

**Keywords:** Xylene-free, Histoprocessing, Microwave temperature, Histological quality.

How to cite:
Eberechukwu et al. 2024. "Xylene-Free Histoprocessing using an Alternative Method: Effect of Microwave Temperature". *Healthcare Studies* 2(1): 33 – 45.
https://doi.org/10.58612/hs214

Received:
November 13, 2023
Accepted:
March 10, 2024
Published:
March 21, 2024

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## Introduction

Microwave energy is a component of the electromagnetic spectrum with a frequency similar to that of radar beam energy (Schwan, 1960). Agitation and heat are used to accelerate the diffusion of solvents in tissue during tissue processing (Bancroft *et al*., 2013). A rise in temperature increases the exchange of fluid and the rate of penetration; however, this must be used in moderation to reduce the chances of introducing heat artefacts into tissue morphology. Extreme heat exposure can cause tissue shrinkage and hardening, which affects staining and immunohistochemistry (Bancroft *et al*., 2019). Techniques for histoprocessing have not changed considerably over the years. The manual steps of dehydration, clearing, and infiltration allow the embedding of tissue samples with a solid support medium such as paraffin wax for easy sectioning.

While, automation reduces the inconveniences encountered in the manual process, the whole process still takes a whole day for paraffin blocks to be produced. Tissue processing steps, such as fixation, dehydration with alcohol grades, and a clearing agent, are achieved by automated transfer of tissues from one reagent to another, as with modern equipment (Leong, 2004).

The principle of microwave processing is based on the rapid dehydration of previously fixed tissue using microwave energy to heat the reagent (alcohol) to a temperature below its boiling point. Isopropanol further dehydrates the tissue and prepares it for paraffin infiltration. The remaining isopropanol is efficiently evaporated using microwave energy to heat (Rohr *et al*., 2001). The rate of penetration and evaporation can be reduced when a thick or fatty tissue is involved, because they contain less water. Substances heat up at different rates even though they are subjected to the same amount of microwave energy. Water, for example, requires higher heat energy to warm up than alcohol of equal volume (Bancroft *et al*., 2008, Shrestha *et al*., 2015). When tissue is immersed in liquid medium, a gradual change in temperature occurs because of the absorption of energy (Shrestha *et al*., 2015). By convention, scientists have adhered to the use of xylene and other clearing agents that have proven to be toxic, however, other alternatives can perform the functions (Leong, 2004).

Even though good sections could be produced from routine overnight processing, it is also a key bottleneck in the workflow of histopathology laboratories. Moving further, the practice is posing some challenges because of its inability to support the increasing clinical demands in health institutions (Smith *et al*., 2012). Specimens such as the liver, spleen, kidney, and heart have been fixed using microwaves as reported by God's man *et al*., 2023. According to Leong (2004), fixation of large specimens, such as colon, lymphoadenectomy, and whole breast specimens, was accomplished by hardening the tissue for embedding and microtomy without exposing the personnel to toxic fumes.

Mac-Moune *et al.* (1987) accounted for suitable outcomes with immunofluorescence in kidney specimens fixed in microwave energy. According to Gown *et al*. (1993), its application in antigen retrieval is innovative with clear expressions in paraffin sections, cytological preparations, and for in situ hybridization (Leong, 2002). In addition, tissues fixed with microwaves showed better antigen preservation than those fixed with formalin (Leong & Milios, 1988). Tissue exposure to microwaves for a short duration recorded a substantial increase in all these procedures, and in many cases, better consistency was produced and histochemical and immunohistochemical staining improved (Leong, 2004).

When tissue preparation is properly controlled, it offers tissues with an uncompromised architecture. Turnaround time is improved even as environmentally friendly reagents are employed as a replacement for those with toxic effects (Bancroft *et al*., 2013). The introduction of this new technique in histology led to a re-evaluation of conventional practice. Various stages of tissue fixation and preparation were made easier in most of the studies, as

reported by various authors (God'sman *et al*., 2023. Boon *et al.,* 1995, Visinoni., 1998). Moreover, regarding tissue structure, staining, and cellular details, the new techniques gave a reliable results with a significant decrease in time. However, there was a marked shrinkage of the tissue bits (Devi, 2013).

Several studies have been conducted to determine how microwaves can replace and/or serve as an alternative method for tissue processing. A study by Alka (2008) revealed that the overall tissue quality was better with one of the rapid methods than the conventional method. However, one protocol recorded a low score for tissue morphology. Schroeder *et al*. (2006) also realized a remarkable decrease in fixation time with the aid of a microwave within 4 hours 25 minutes and 3 hours 20 minutes in two rapid protocols. On electron microscopy (EM), sections were stable and image quality was uniform. However, unlike the conventional automatic tissue processor, transfer of specimen is done manually using the microwave processor (Schroeder *et al*., 2006).

The present study addresses the negligence of the effect and efficiency of microwave energy in histological techniques. This alternative method has more than a few merits over conventional methods from the viewpoint of laboratory staff. It also has some environmental benefits as it eliminates xylene in tissue processing. It may also decrease or completely eradicate the choice for formalin, in the laboratory (Rohr *et al*., 2001). Changing the technique for histoprocessing from the currently used overnight processing to same-day microwave tissue processing could significantly reduce turnaround times, (Tripathi *et al*., 2013, God'sman *et al*., 2023), which permits same-day analysis that would improve patient diagnosis, management, and research (Babu *et al*., 2011, Rohr *et al*., 2001). Therefore, the present study assessed the product of xylene-free tissue processing. This study analysed the histological quality of tissues in different schedules based on cellular clarity. The mean difference between the scores allotted to the products of the control method and the rapid protocols was considered using ANOVA. Similarly, to determine the effect of microwave temperature on histomorphology.

## Materials and Methods

The present study was conducted at the Federal University Otuoke, Nigeria. The study was based on xylene-free tissue processing using an alternative method; the effect of microwave temperature. Fresh tissues (kidney, liver, heart, and intestine) from Albino Wistar rats were subjected to the rapid protocol. Processing was performed in a kitchen microwave, model MW007GA-MG823 (W)/MW-MB0823MP, rated voltage: 230V5~0HZ. Specimens were processed using four (4) rapid protocols. Each organ was divided into five parts. The first, second, third, and fourth parts of each were processed using the designed rapid protocols and the $5^{th}$ part was processed using the traditional method. Processing of tissues with the alternative protocol was performed with thorough attention to determine the efficiency and effectiveness of the kitchen microwave in executing tissue processing without employing xylene.

**Sample Selection**

Samples for this study were obtained from five (5) healthy Albino wistar rats. It was ensured that the subjects were in good condition before they were sacrificed. The specimens were immediately placed in 10% formal saline after resection to avoid autolysis and/or putrefaction. The specimens used for this study included the spleen, liver, kidney, heart, intestine, and lungs.

**Rapid protocol**

In the first and second rapid protocols, fixation was performed in the kitchen microwave oven within 1500 seconds at a temperature of 323K and 5319k at 1800 seconds respectively. Further analysis with a different protocol, as shown in table 1, was performed at the temperature of 315K for dehydration and 4523K for infiltration. Clearing agents were not employed because the temperatures of the final paraffin step increases the evaporation of alcohols from the tissue.

Following infiltration, molten paraffin wax was poured into embedding moulds and forceps were used to transfer the tissue into the mould. The tissue was oriented in position, making the surface that would be cut to face downward. The base of the cassette was placed on the mould, and molten paraffin wax was poured on the cassette until the mould and the cassette were completely filled. The mould was transferred to ice for solidification of the block. The block was then detached from the mould through a rigid support of paraffin wax.

**Table 1.** Microwave Protocol

| Reagent | Temperature | Time |
|---|---|---|
| 10% formal saline | 298K | 32400 seconds |
| 10% formal saline (rapid) | 315K | 1200 seconds |
| 70% alcohol | 315K | 900 seconds |
| 90% alcohol | 315K | 900 seconds |
| Absolute alcohol | 315K | 900 seconds |
| Paraffin wax 1 | 4523K | 1200 seconds |
| Paraffin wax 2 | 4523K | 1200 seconds |

Microtomy was performed using a manual Rotary microtome, and sections of 5 micrometer were cut. Microtomy was succeeded by routine manual haematoxylin and eosin staining technique for the demonstration of general tissue architecture. Sections

were immersed in two changes of xylene for 10 minutes each. The sections were gradually hydrated from descending grades of alcohol down to water. Slides were then transferred to a trough of haematoxylin stain solution for 5 minutes and then rinsed in water. Sections were briefly differentiated in 1% acid alcohol and subsequently rinsed in water. They were then transferred to a trough of 1% alcoholic eosin for 2 minutes. Slides were washed in water, dehydrated with ascending grades of alcohol up to absolute alcohol, and mounted with dibutyl phthalate polystyrene xylene (DPX) mountant. (Bancroft & Gamble 2008, Mahesh Babu *et al*., 2011, Culling's *et al*., 1985).

The sections produced from each protocol were graded with numerical values with the following parameters: cellular clarity, nuclear details, colour intensity, and thickness of sections. Sections were assessed macroscopically and microscopically.

## Results

This study examined xylene-free histoprocessing using an alternative method: the effect of microwave temperature. The methodology involved a traditional scheme and an alternative protocol. The investigation revealed the findings expressed below.

Table 2 shows the mean andstandard deviations of the scores allotted to the tissue sections in each of the alternative protocol and the traditional processing. Protocol 1 (Mean±SD =1.8000±.83666), protocol 2 (Mean±SD = 4.4000±.89443), protocol 3 (Mean±SD =2.2000±1.30384), and protocol 4 (Mean±SD = 4.4000±1.14018) while the mean and standard deviation of the control sections are shown as (Mean±SD= 6.6000±.89443). Table 2 also presents a one-way AVOVA – F(24) = 17.792, p = 0.00, which is statistical significant. The result discloses significance at $p<0.05$, which implies that the mean scores of the tissue sections in the experimental protocols and the traditional protocols are different.

**Table 2.** Mean, standard deviation and ANOVA of the tissue scores in each protocol across various organs based on cellular clarity, nuclear details, and colour intensity.

| Method | N | Mean±SD | F-value | Df | p-value |
|---|---|---|---|---|---|
| Protocol 1 | 5 | 1.8000±.83666 | | | |
| Protocol 2 | 5 | 4.4000±.89443 | | | |
| Protocol 3 | 5 | 2.2000±1.30384 | 17.792 | 24 | 0.00 |
| Protocol 4 | 5 | 4.4000±1.14018 | | | |
| Traditional (Control) | 5 | 6.6000±.89443 | | | |
| Total | 25 | 3.8800±2.00666 | | | |

Table 3 shows Turkey's posthoc test of multiple comparisons between the experimental protocols (PRT 1, PRT 2, PRT 3, PRT 4). This established that the mean

scores of the tissue sections from the various protocols were statistically different ($p<0.05$).

**Table 3.** Turkey Posthoc Test - Multiple Comparisons of Mean Scores of Rapid Protocols and Control group.

| (I) Method | (J) Method | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|
| PRT 1.00 | PRT 2.00 | -2.60000* | .65115 | .001 |
| | PRT 4.00 | -2.60000* | .65115 | .001 |
| | CONTROL | -4.80000* | .65115 | .000 |
| PRT 2.00 | PRT 1.00 | 2.60000* | .65115 | .001 |
| | PRT 3.00 | 2.20000* | .65115 | .003 |
| | CONTROL | -2.20000* | .65115 | .003 |
| PRT 3.00 | PRT 2.00 | -2.20000* | .65115 | .003 |
| | PRT 4.00 | -2.20000* | .65115 | .003 |
| | CONTROL | -4.40000* | .65115 | .000 |
| PRT 4.00 | 1.00 | 2.60000* | .65115 | .001 |
| | 3.00 | 2.20000* | .65115 | .003 |
| | CONTROL | -2.20000* | .65115 | .003 |
| CONTROL | 1.00 | 4.80000* | .65115 | .000 |
| | 2.00 | 2.20000* | .65115 | .003 |
| | 3.00 | 4.40000* | .65115 | .000 |
| | 4.00 | 2.20000* | .65115 | .003 |

*. The mean difference is significant at the 0.05 level.

Table 4 reveals the mean, standard deviation, and ANOVA result for each tissue score across the rapid protocols. The investigation shows that kidney has mean±SD = 4.2000±1.48, liver (mean±SD = 4.4000±2.88), spleen (mean±SD= 4.4000±1.34), intestine (mean±SD = 2.8000±2.05). Moreover, the ANOVA result across the alternative protocol is as follows $F(24) = 0.541$, $P = 0.707$. This proves a non-statistical significance at $P<0.05$, hence the quality of the distinct organs (histologically) is not different across the rapid protocols employed.

**Table 4.** Mean and standard deviations of tissue Scores across rapid protocols

| Tissue | N | Mean±SD | Df | F | P-value |
|---|---|---|---|---|---|
| Kidney | 5 | 4.2000±1.48 | | | |
| Liver | 5 | 4.4000±2.88 | | | |
| Spleen | 5 | 4.4000±1.34 | 24 | .541 | .707 |
| Heart | 5 | 3.6000±2.30 | | | |
| Intestine | 5 | 2.8000±2.05 | | | |
| Total | 25 | 3.8800±2.01 | | | |

A comparative analysis between the conventional and microwave techniques shows slight evidence of agreement with a kappa value of 0.00. See table 5 for details.

**Table 5.** Kappa measure of agreement between the rapid protocols and the control group.

| Method | Kappa Value | Remark |
|---|---|---|
| Control vs. Protocol 1 (PRT1) | 0.000 | Slight Agreement |
| Control vs. Protocol 2 (PRT2) | 0.003 | Slight Agreement |
| Control vs. Protocol 3 (PRT3) | 0.000 | Slight Agreement |
| Control vs. Protocol 4 (PRT4) | 0.003 | Slight Agreement |
| Protocol 1 (PRT 1) vs. Protocol 2 (PRT 2) | 0.001 | Slight Agreement |
| Protocol 1 (PRT 1) vs. Protocol 4 (PRT 4) | 0.001 | Slight Agreement |
| Protocol 2 (PRT 2) vs. Protocol 3 (PRT 3) | 0.003 | Slight Agreement |
| Protocol 3 (PRT 3) vs. Protocol 4 (PRT 4) | 0.000 | Slight Agreement |

Note: <0 = No agreement; 0.0 - 0.2 = Slight agreement; 0.21 - 0.4 =Fair agreement; 0.41 - 0.6 = Moderate agreement; 0.61 - 0.8 = Substantial agreement; 0.81 - 0.99 = Almost perfect agreement; 1 = Perfect agreement.

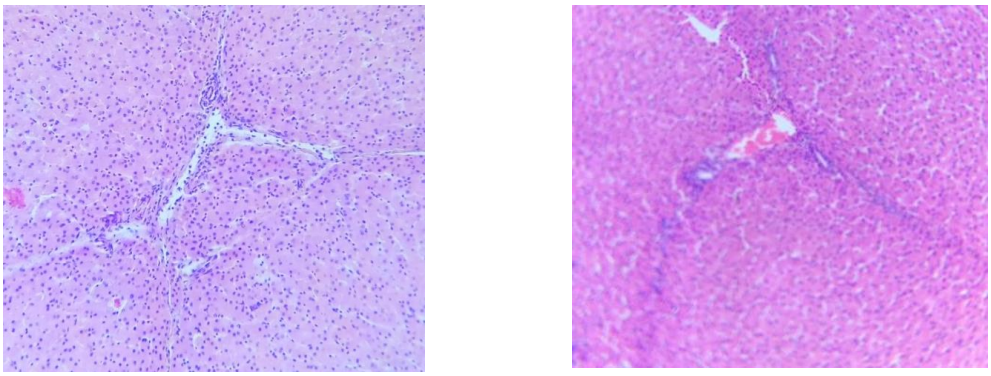
A bivariate regression was conducted to examine the effect of temperature on tissue score. From, table 6, the correlation between temperature and tissue score is shown as $r(23) = 0.259$, $P > 0.05$. The regression equation for predicting the tissue score from temperature was $\hat{Y} = 4.960 + (-0.360x)$. The $R^2$ for this equation is 0.067; that is, 6.7% of the variance in tissue score was predicted by temperature. The boot strapped 95% confidence interval for the slope to predict tissue score from the temperature range from -0.939 to +0.219. Consequently, for each unit increase in temperature, cellular clarity decreased by approximately 0.2 - 0.9 points.

**Table 6.** Regression analysis showing the effect of microwave temperature on histomorphology.

| Regression Weights | | Beta Coefficient | $R^2$ | F | P-value |
|---|---|---|---|---|---|
| **Temperature** | **Tissue** | -.259 | 0.067 | 1.653 | 0.211 |

**Table 7.** Summary of the Quality of Tissues Processed using the Rapid Protocol and the Problems Encountered.

| Specimen type | Specimen Quality | Problem encountered |
|---|---|---|
| Liver | Suboptimal | Poor fixation, suboptimal clarity, and cellular detail. |
| Spleen | Suboptimal | Suboptimal fixation, |
| Lungs | Suboptimal | Darkened smudged center of the tissue suggestive of less penetration. Sections too thick |
| Heart | Suboptimal | Fragmented tissue Suboptimal clarity and cellular detail |
| Kidney | Unsatisfactory | Less optimal nuclear and cytoplasmic details. |
| Intestine | Suboptimal | Tissue not hard enough for sectioning; hence, sections were poor. Sections were lifted from slides during staining. suboptimal clarity and cellular detail. |



**A** **B**

**Figure 1.** Liver sections (×10 objective). A is from traditional processing, and B from the alternative method.

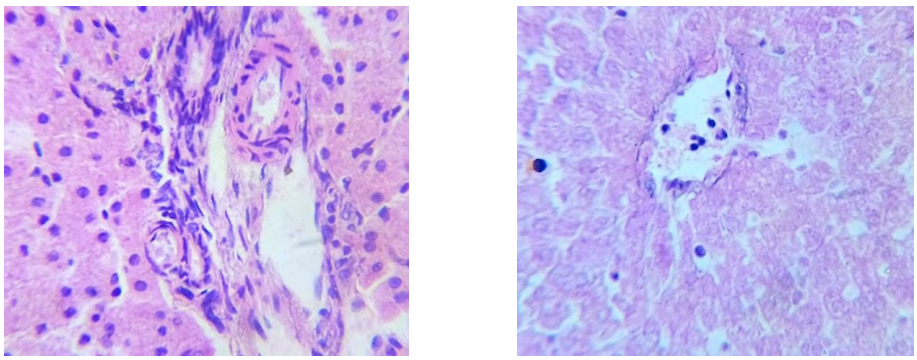



**Figure 2.** Liver sections (×40 objective). A is from traditional processing, while B from the alternative method.

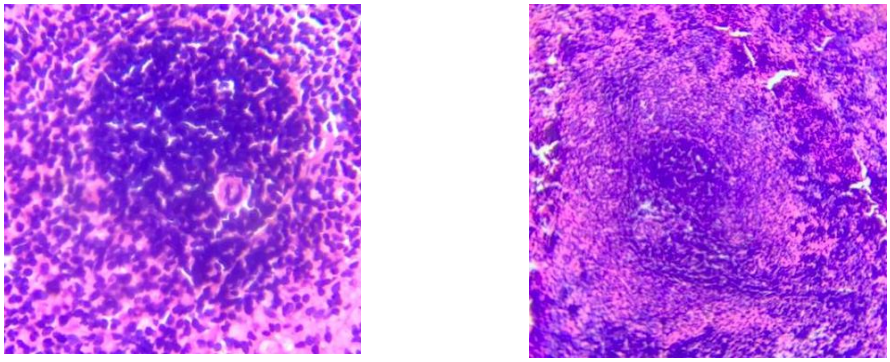



**Figure 3.** Sections of the spleen. A was processed using the traditional method, while B was processed using the alternative method.

## Discussion

Advances have been made in the understanding of tissue morphology in normal and disease conditions. The management of tissue samples from surgical removal to processing and staining has gained some scientific advances (Babu *et al*., 2011). However, formalin fixation and traditional tissue processing techniques have been the gold standard for many years (http://www.ebsciences.com/papers/microwaves.htm). Exposure to microwave energy stimulates the diffusion of reagents into the tissue by increasing the internal heat of the specimen, thereby speeding up the reaction rate (Bancroft *et al*., 2013).

According to Patil *et al*. (2014), the stages of traditional histoprocessing are complex, whereas microwave techniques are easier and make use of isopropanol, which serves a dual purpose as a dehydrating and clearing agent. The residual alcohol evaporates using microwave energy during impregnation, thereby eliminating the separate clearing procedure. This in turn slashes the cost, materials, and toxic effect of xylene (Babu *et al*., 2011, and Panja *et al*., 2007).

For the first objective, which is to assess the inter-ratal agreement between the routine and alternative methods, the kappa measure of agreement revealed slight evidence of

agreement with a kappa value of 0.00, as shown in table 5. Also, in objective 2 (two), cellular clarity in all the protocol was evaluated; nuclei and cytoplasm of cells were visible, but sections from the traditional method were better. Staining was fairly sharp in some of the sections, although some sections were lost. However, poor fixation, suboptimal clarity, and cellular detail were detected. See the photomicrographs of the sections in figure 1 and 2 above. In a study by Patil *et al*. (2014), epithelial tissue processed and stained by the microwave method showed better quality of cellular and nuclear details with prominent intercellular bridges. Adipose tissue and muscle tissue also showed good preservation of cellular and nuclear details in tissues processed and stained by the microwave method. Consequently, it was confidently stated that the use of microwave processing and staining methods for the aforementioned tissues was better (Patil *et al*., 2014). In addition, in a study by Panja *et al*. (2007), loss of nuclear details under high power objective was observed when histo-processing was completely performed in the microwave. In contrast, in different studies by Kennedy & Foulis (1998) and Reed *et al*.(1991), it was established that the cytological details of the nuclei were much clearer using the microwave technique. In addition, comparative analysis between the alternative method and the traditional technique on the basis of cellular clarity, colour intensity, and nuclear details showed statistically significance, as shown in table 2 and 4.

Furthermore, in a study by Smith et al. (2012), on the comparison of clarity of nucleocytoplasmic differentiation of oral tissues processed by microwave and conventional methods, scores were given using the same parameters. The study revealed 6 different protocols that were not statistically significant. Fibrous tissue in protocol 5 of the study revealed better clarity. There was a statistically significant difference between the protocols. The scores given for clarity of nucleocytoplasmic differentiation or integrity of epithelial tissues after being processed by 6 different protocols were not statistically significant (Table 2), whereas protocol IV revealed better clarity for fibrous tissues (Table 3). There were no significant differences among different protocols when considering the clarity of nucleocytoplasmic integrity differentiation or integrity of glandular and muscle tissues (Table 4, Table 5)

Shrestha *et al*., (2015) also stated that cellular clarity. Colour and overall staining quality were better for the microwave technique, and there was no statistical significance between the two methods. In addition, the cytoplasmic and nuclear details were interpreted as good to excellent after examination. However, the study recorded a significant difference between the two techniques at $\alpha < 0.05$, whereas the conventional method showed equal or slightly higher mean scores. The mean scores for cellular clarity, colour intensity and overall staining quality were slightly higher for the microwave method, but no statistically significant variation was observed between the two methods $\alpha < 0.05$ (Shrestha *et al*., 2015).

Similarly, God'sman *et al*., 2023 reported that sections produced by the microwave were almost equal to those processed with the traditional tissue processor. Mayers, (1970), also a pioneer researcher in microwave tissue processing, reported that the nuclear structure was in good shape, with well preserved cytoplasm. It was also reported that the brush borders of the epithelium lining the tubules were-preserved. However, the loss of red blood cells as a result of lysis and a smaller amount of

collagen fibers after fixation was a drawback in the study. Moreover, better architectural details of adipose tissues were noted by Tripathi *et al*., 2013 after microwave fixation. However, there was some loss of tissues, as reported by Tripathi *et al*., (2013), especially tissues such as the cervice, gall bladder, lymphoid, and tumour tissues. Conversely, Morales *et al*. (2022), could not record any loss of tissue architecture between the two methods.

For objective 3, the mean difference between the grades of the experimental group and the traditional method was investigated using test ANOVA, which revealed the following results: Protocols 1, 2, 3, and 4 (PTR1, PTR2, PTR3, PTR4) recorded a mean and standard deviation of (Mean±SD =1.8000±.83666), (Mean±SD = 4.4000±.89443), (Mean±SD =2.2000±1.30384), and (Mean±SD = 4.4000±1.14018) respectively. The traditional method recorded (Mean±SD= 6.6000±.89443). In addition, the ANOVA result showed a statistically significant value of F(24) = 17.792, p = 0.00, as shown in Table 2. However, Table 4 shows no statistical significance between the rapid protocols with an ANOVA result of F(24) = 0.541, P = 0.707. This is similar to a study by Rao *et al*., 2020, who affirmed that cellular clarity and epithelium and connective tissue interface were statistically significant. The study by Rao *et al*., 2020 revealed that cellular clarity and epithelium and connective tissue interface were better in tissues processed by the conventional method than microwave processing.

Furthermore, to determine the effect of microwave temperature on histo morphology, a linear regression analysis was conducted to examine the effect of temperature on tissue morphology. From, table 6, $R^2 = 0.067$ p = 0.21, which implies that 6.7% of the variance in tissue morphology was predicted by the temperature of the microwave, and the temperature non-significantly predicted tissue morphology.

The rapid method of histoprocessing will positively impact the advancement of histopathology. This alternative protocol responds quickly to laboratory needs, creating a staff and/or environment workflow (Smith *et al*., 2012). The drawback of this technique is that the process could be laborious because the reagents are manually manipulated, and the temperature must be maintained at a particular range. Moreover, the cost of laboratory-grade microwaves may be exorbitant, and proper use of the microwave oven requires careful calibration and monitoring (Bancroft *et al*., 2013).

## Conclusion/Recommendation

Using microwaves in histopathology laboratories stimulates the rate of tissue processing because of heat energy. This makes the xylene-free technique worthwhile in cases where rapid diagnosis is needed and a frozen section is uncertain. The use of toxic chemicals could also be avoided. The specific materials appropriate for use in the microwave are plastics and plain glassware. This is because microwaves cannot pass through metallic materials. The energy and time of irradiation should be regulated/managed properly to avoid harm. This method is capable of aiding in treating patients in emergencies. Domestic microwave ovens without calibrations may not be suitable for use because the irradiation power may not be established to help in regulate the temperature.

**Study Limitations:**

A kitchen microwave was used for the investigation instead of a laboratory grade microwave, and efforts to calibrate it proved to be abortive.

**Conflicts of Interest:** None.

## References


[1] Alka Mary Mathai, Ramadas Naik, Muktha R Pai, Sharada Rai, Poornima Baliga. (2008). Microwave Histoprocessing Versus Conventional Histoprocessing. Indian Journal of Pathology and Microbiology Volume (51) pg: 12-16 Available from: https://www.ijpmonline.org/text.asp?2008/51/1/12/40383

[2] Babu T.M., Malathi N., Agesh K.T. A. (2011). Comparative Study on Microwave and Routine Tissue Processing. Indian Journal of Dental Research, (22): 50-55.

[3] Bancroft J.D, S. Kim Suvarna, Christopher Layton (2019). Bancroft's Theory and Practice of Histological Techniques. 8th ed. Elsevier.

[4] Bancroft JD, Gamble M. (2008). Bancroft's Theory and Practice of Histological Techniques. 7th ed. Kim S Suvarna, S. Kim Suvarna, Christopher Layton JDB, editor. Elsevier Health Sciences.

[5] Boon M.E, Wals-Paap C.H., Visinoni F.A, Kok L.P. (1995). The Two-Step Vacuum-Microwave Method. Microwave Newsletter 13. Eur J Morphol. Vol (33):349-58.

[6] Gown AM, de Wever N, Battifora H. (1993). Microwave-based antigenic unmasking: a revolutionary new technique for routine immunohistochemistry. Appl Immunohistochem.;1:256-266.

[7] John D.B, S. Kim Suvarna Christopher Layton (2013). Theory and Practice of Histological Techniques.7th ed., Elsevier Churchill Livingstone.

[8] Kennedy A, Foulis AK. (1998). Use of microwave oven improves morphology and staining of cryostat sections. Journal of Clinical Pathology;42:101-5.

[9] Leong AS-Y. (2004). Microwaves and Turnaround Times in Histoprocessing: This a New Era in Histotechnology? Am J Clin Pathol; 121:460-2.

[10] God'sman C. Eberechukwu, Amalachukwu Ike, Gift Okwusike, Ibiere pepple (2023). The Effect of Rapid Tissue Processing on Fixation Time Using Microwave Techniques. Journal of Applied Health Sciences and Medicine 3(1): 10-20.

[11] Leong AS-Y, Milios J. (1988). Antigen preservation in microwave- irradiated tissues: a comparison with formaldehyde fixation. J Pathol.;156:275-282

[12] Leong AS-Y, Milios J. (1993) An assessment of the efficacy of the microwave antigen-retrieval procedure on a range of tissue antigens. Appl Immunohistochem; 1:267-274.

[13] Leong AS-Y, Leong FJW-M. Microwave-stimulated antigen retrieval: an update. Acta Histochem Cytochem. 2002;35:367-374.

[14] Leong, A. S. (2004). Microwaves and turnaround times in histoprocessing. Am J Clin Pathol, 121, 460-462.

[15] Mayers C.P. (1970). Histological Fixation by Microwave Heating. Journal of Clinical Pathology, 23(3), 273-275. Doi: 10.1136/jcp.23.3.273.

[16] Mac-Moune LF, Lai KN, Chew EC, & Lee, J. C. (1987). Microwave Fixation in Diagnostic Renal Pathology. Pathology, 19(1), 19:17-21. https:// doi.org/10.3109/003130287090651.

[17] Microwave Processing Technique for Microscopy. Available from: http://www.ebsciences.com/papers/microwaves.htm

[18] Morales AR, Essenfeld H, Essenfeld E, Duboue MC, Vincek V, Nadji M. (2002). Continuous-Specimen-Flow, High-throughput, 1-Hour Tissue Processing. Archives Pathol Lab Med. 126:583-90.

[19] Panja P, Sriram G, Saraswathi TR, Sivapathasundharam B. (2007). Comparison of three different methods of tissue processing. J Oral Maxillofac Pathol;11:15-7.

[20] Patil S, Rao RS, Nagaraja A, Kumar SD. (2014). Comparison of conventional and microwave histo-processing of various oral soft tissue specimens. Journal of Dental Research Rev; 1:3-6. https://www.jdrr.org/text.asp?2014/1/1/3/126154

[21] Reed W, Erichsen A, Roald B. (1991). Rapid supplementary fixation in frozen sections: Microwave versus conventional fixation: A double blind comparative study. Pathology Research and Practice. ;187(7):824-7.

[22] Rao, M., Pai, S. M., Khanagar, S. B., Siddeeqh, S., Devang, D. D., Naik, S. (2020). Microwave-Assisted Tissue Processing, Fixation and Staining in Tissues of Different Thicknesses: A Comparative Study. Journal of oral and maxillofacial pathology: JOMFP, 24(1), 186. https://doi.org/10.4103/jomfp.JOMFP_290_19
[23] Rohr L.R, Layfield L.J, Wallin D. Hardy D, (2021). A Comparison of Routine and Rapid Microwave Tissue Processing in a Surgical Pathology Laboratory. Quality of Histologic Sections and Advantages of Microwave Processing. Aerican Journal of Clinical Pathology; 115: 703- 708.
[24] Schroeder, J. A., Gelderblom, H. R., Hauroeder, B., Schmetz, C., Milios, J., & Hofstaedter, F. (2006). Microwave-assisted Tissue Processing for Same-day EM-diagnosis of Potential Bioterrorism and Clinical Samples. Micron (Oxford, England: 1993), 37(6), 577–590. https://doi.org/10.1016/j.micron.2005.11.015
[25] Schwan, H. P. (1960). In Medical Physics, Vol. 3. Edited by 0. Glasser. Year Book Inc, Chicago, Ill.
[26] Shrestha, G., Karki, S., & Pradhan, A. (2015). Changing perspective on tissue processing-comparison of microwave histoprocessing method with the conventional method. Journal of Pathology of Nepal, 5(10), 841-846.
[27] Smitha Naik K., Shilpa Patel, Madhusudhan Astekar, Dinesh Rao (2012). Comparison of Clarity of Nucleocytoplasmic Differentiation of Oral Tissues Processed by Microwave and Conventional Methods. Annals of Diagnostic Pathology. Vol:16, Pg 128-133. https://doi.org/10.1016/j.anndiagpath.2011.07.008
[28] Panja P, Sriram G, Saraswathi TR, Sivapathasundharam B. (2007). Comparison of three different methods of tissue processing. J Oral Maxillofac Pathol. ;11:15-7.
[29] Tripathi, M., Bansal, R. Gupta, M., Bharar, V. (2013). Comparison of Routine Fixation of Tissues with Rapid Tissue Fixation of tissues with rapid Tissue Fixation. Journal of clinical and diagnostic Research: JCDR, 7(12). 2768-2773. DOI: 10.7860/JCDR/2013/6233.375
[30] Visinoni F, Milios J, Leong AS-Y, et al. Ultra-rapid microwave/variable pressure induced histoprocessing: description of a new tissue processor. J Histotechnol. 1998; 21:219-224.